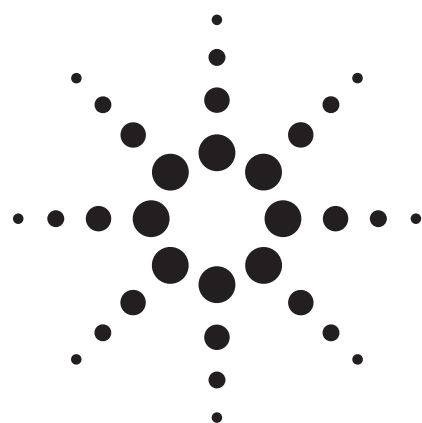


Analysis of Natural Food Colorants by Electrospray and Atmospheric Pressure Chemical Ionization LC/MS



Application

Food

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Originally published April 2000

Abstract

Four commercial natural colorants – red cabbage, *Monascus*, paprika, and lac – were analyzed using ESI and APCI-LC/MS. The MS data provided molecular weight information and some structural information for the major components in these pigments.

Introduction

Many kinds of natural colors are used in beverages, jellies, and candies. In many countries, food regulations have been recently revised to cover natural colorants to the same degree as synthetic ones. Accordingly, it has become necessary to develop reliable analytical methods for various natural colorants in food. In this study, LC/MS methods using electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI) were developed to identify major pigments in four natural colorants: red cabbage, paprika, *Monascus*, and lac.

Experimental

Paprika and *Monascus* colorants were dissolved in acetone; the other colorants were dissolved in

deionized water. Each colorant was filtered through a 0.2- μ m filter. A 10- μ L portion was injected into the system, which consisted of an Agilent 1100 Series binary pump, thermostatted column compartment, vacuum degasser, autosampler, and LC/MSD. The LC/MSD used either an ESI or APCI source. Complete system control and data handling were done on the Agilent ChemStation for LC/MS. Operating conditions were optimized for each sample.

Results and Discussion

Red Cabbage Colorant

Figure 1 shows the structure of seven major pigments of red cabbage. The pigments share the basic cyanidin 3-diglucoside structure with differing R1 and R2 groups. Figure 2 shows the total ion chromatogram (TIC) and extracted ion chromatograms (EICs) of red cabbage pigments. Although every major pigment can be chromatographically separated using 10% formic acid in the mobile phase, the high acid concentration reduces sensitivity. Therefore, 1% formic acid was used in this study. The EICs show the separation of the pigments based on their main ion (base peak).

Figure 3 shows the mass spectra of the seven major pigments in red cabbage colorant. For these pigments, the singly charged molecular ion is



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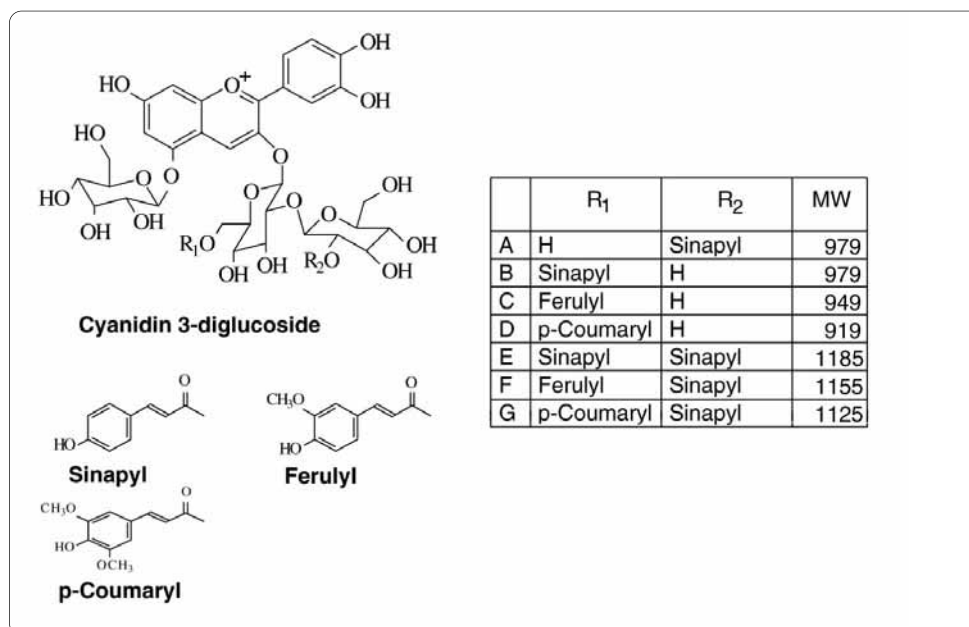


Figure 1. The structure of major pigments in red cabbage colorant.

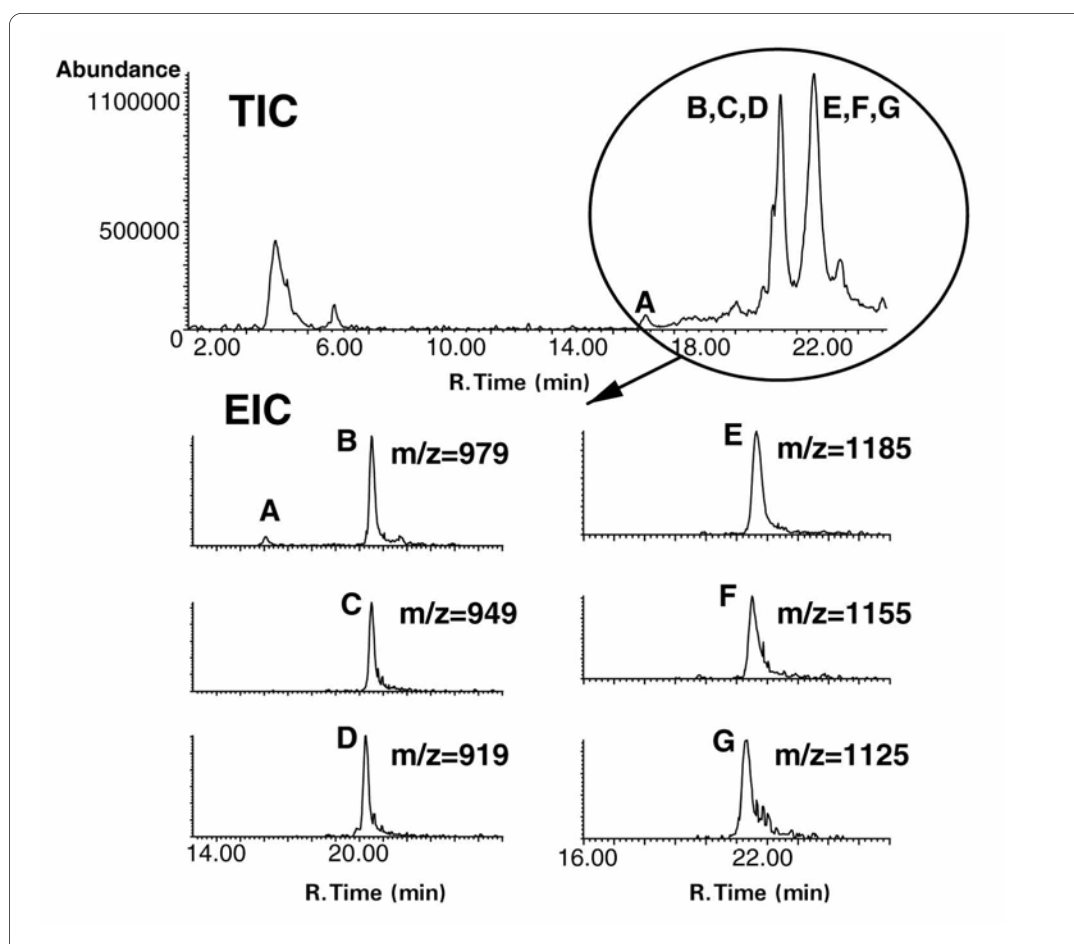


Figure 2. Total and extracted ion chromatograms of red cabbage colorant.

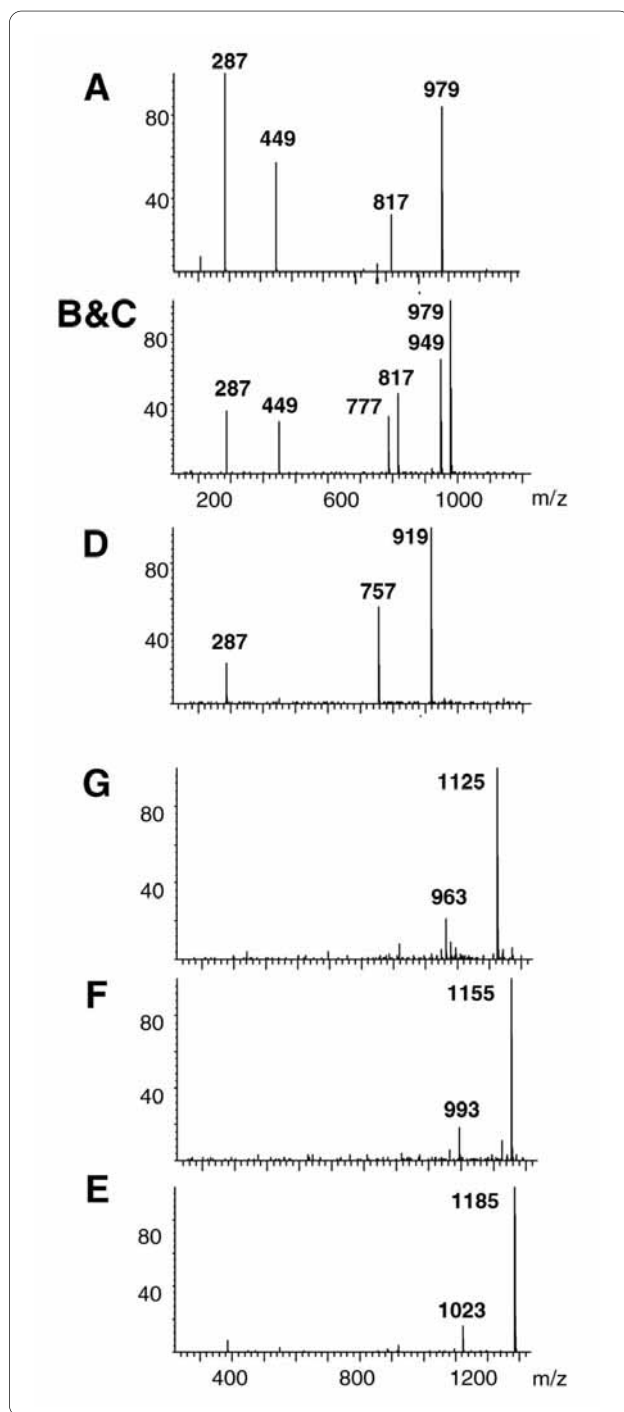


Figure 3. Mass spectra of major pigments in red cabbage colorant.

LC Conditions

Column: Inertsil ODS3, 250 mm × 2.1 mm, 5 μm
 Mobile phase: A = 1% formic acid
 B = acetonitrile
 Gradient: Start with 5% B
 At 30 min 50% B
 Flow rate: 0.2 mL/min
 Column temperature: 40 °C
 Injection volume: 10 μL

MS Conditions

Source: ESI
 Ion mode: Positive
 V_{cap} voltage: 4000 V
 Nebulizer: 50 psig
 Drying gas flow: 10 L/min
 Drying gas temp: 350 °C
 Corona: 4 μA
 Vaporizer temperature: 350 °C
 Scan range: 100–1200 amu
 Step size: 0.1
 Peak width: 0.15 min
 Time filter: On
 Fragmentor: 200 V

observed rather than the more typical $[M+H]^+$ ion, because the cyanidin group already has a positive charge on an oxygen. In-source collision-induced dissociation (CID) can be used to generate fragment ions to provide structural confirmation. Using CID, mass spectra of these pigments show common fragments corresponding to the loss of a glucose, as well as cyanidin (m/z 287) and cyanidin 3-glucoside (m/z 449) ions.

Monascus Colorant

Monascus contains six major pigments; their structures are shown in Figure 4. Four pigments were identified from the mass spectra of major peaks in the TIC. See Figure 5.

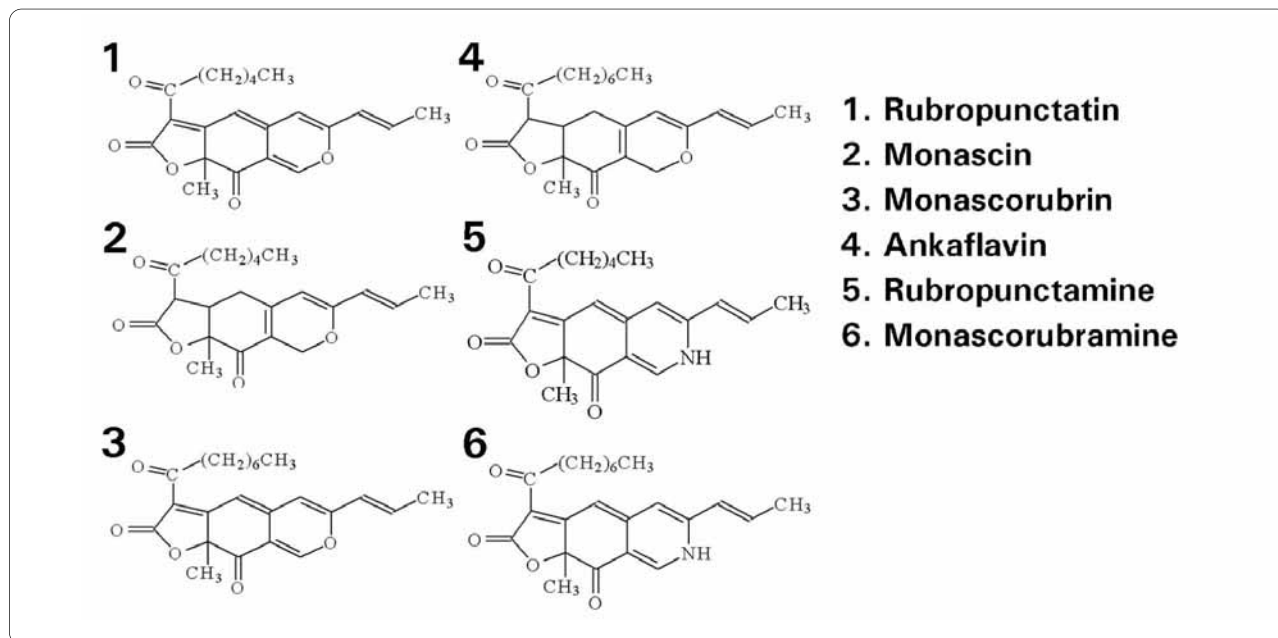


Figure 4. The major pigments of *Monascus* colorant.

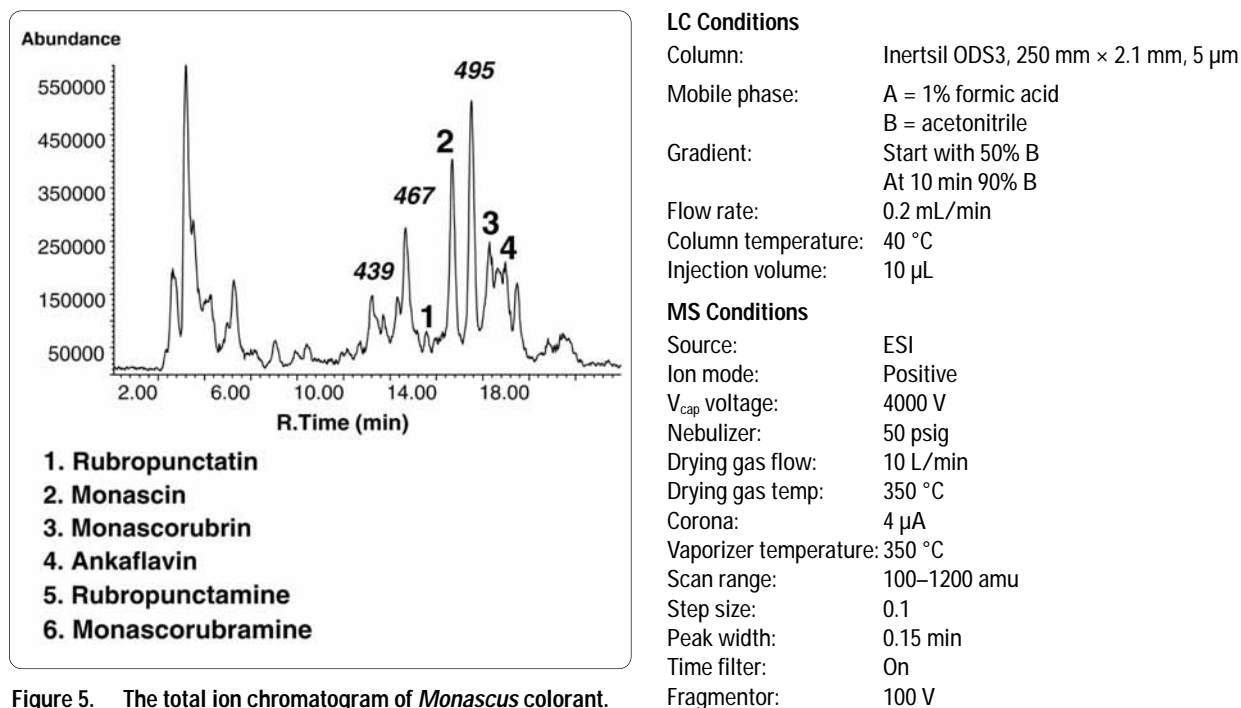


Figure 5. The total ion chromatogram of *Monascus* colorant.

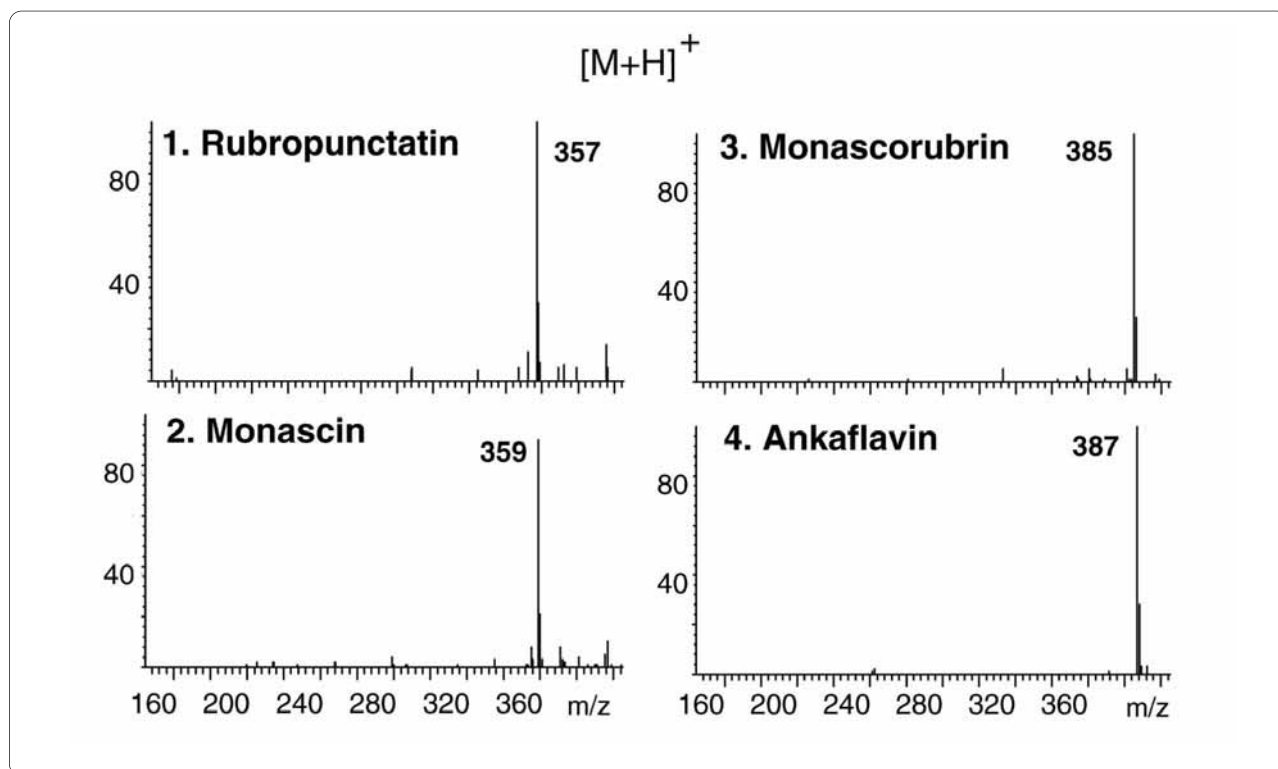


Figure 6. Mass spectra of major pigments in *Monascus* colorant.

Three major peaks with base peaks at m/z 439, 467, and 495 were not identified. Figure 6 shows the mass spectra of the identified pigments. Protonated molecular ions $[M+H]^+$ were observed for the four identified pigments.

Paprika Color

Capsanthin and the mono- and di- esters of capsanthin with fatty acids are known as the major pigments in paprika colorant. See Figure 7. Two monoesters and five diesters of capsanthin were identified in the paprika colorant analyzed in this study. See Figure 8.

The protonated molecular ions $[M+H]^+$ were observed for every major pigment. See Figure 9. However, with the exception of capsanthin monoecisanoate, the intensity of these ions was very low. Except for capsanthin monoecisanoate, the pigments show fragment ions resulting from the loss of one or two fatty acid fragments. A common fragment ion was observed at m/z 567 in the mass spectra of these pigments.

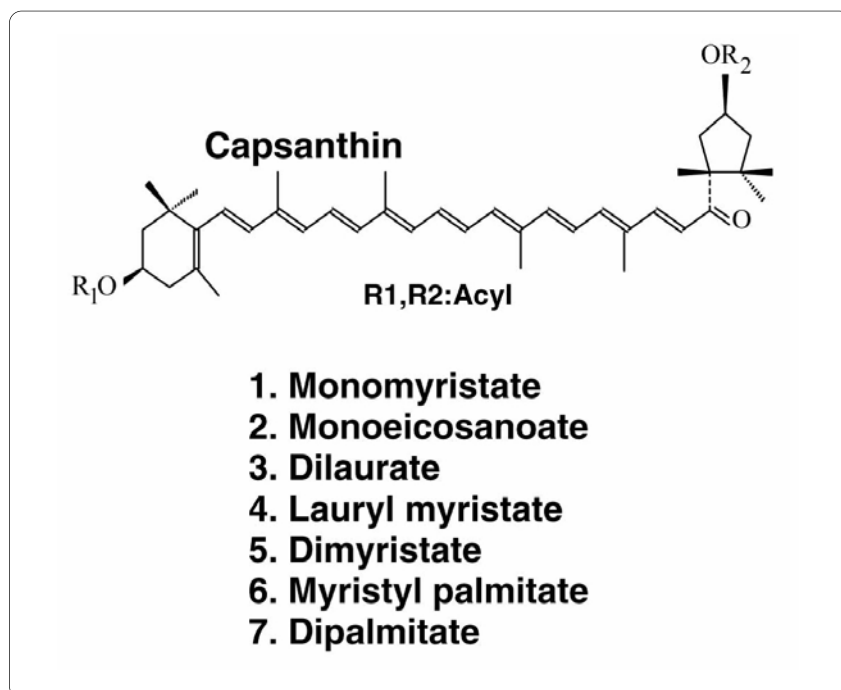


Figure 7. The structure of major pigments of paprika colorant.

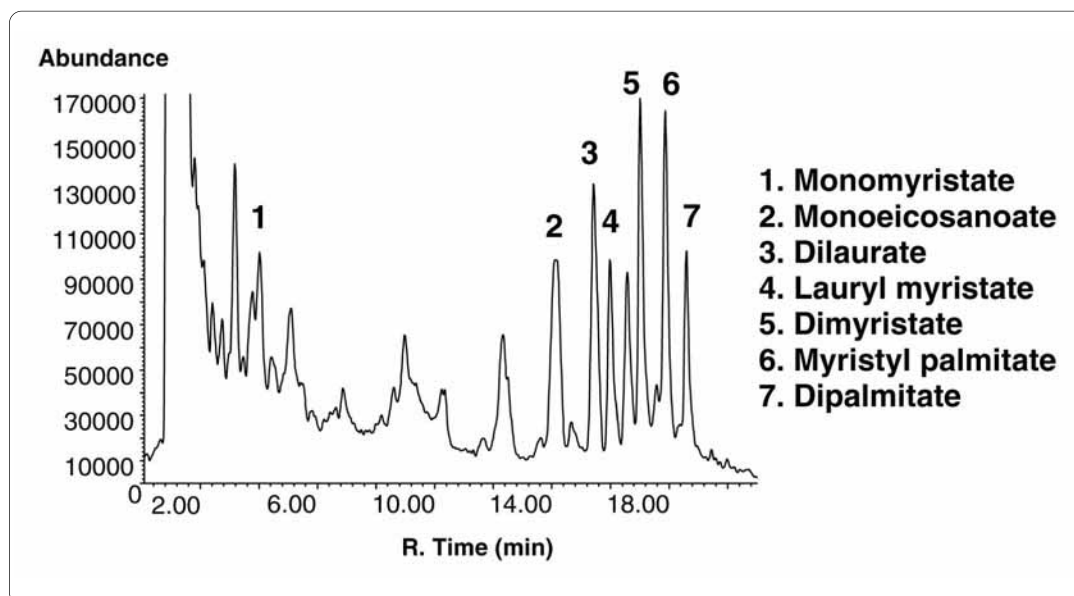


Figure 8. The total ion chromatogram of paprika colorant.

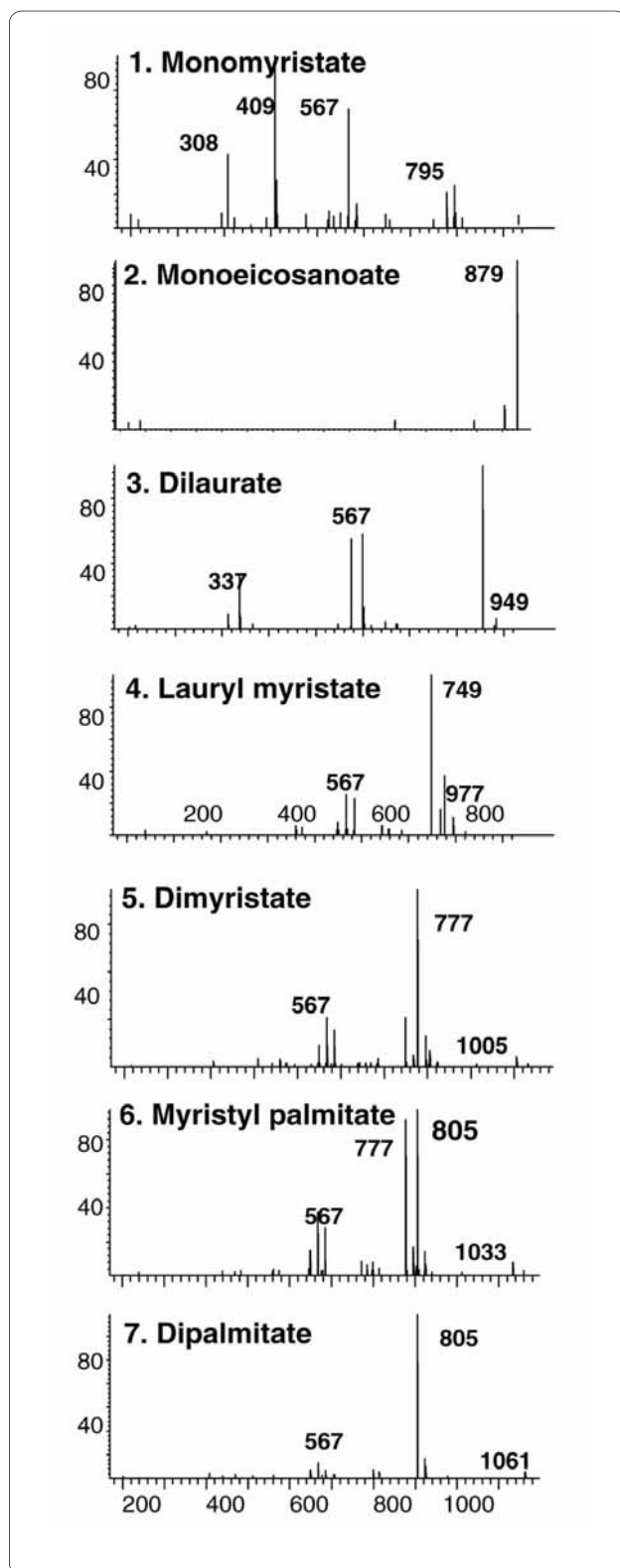


Figure 9. Mass spectra of major pigments in paprika colorant.

LC Conditions

Column: Inertsil ODS3, 250 mm × 2.1 mm, 5 μm
 Mobile phase: A = acetone
 B = methanol
 Gradient: Start with 10% B
 At 10 min 90% B
 Flow rate: 0.2 mL/min
 Column temperature: 40 °C
 Injection volume: 10 μL

MS Conditions

Source: APCI
 Ion mode: Positive
 V_{cap} voltage: 4000 V
 Nebulizer: 50 psig
 Drying gas flow: 5 L/min
 Drying gas temp: 350 °C
 Corona: 4 μA
 Vaporizer temperature: 350 °C
 Scan range: 100–1200 amu
 Step size: 0.1
 Peak width: 0.15 min
 Time filter: On

Lac Colorant

Figure 10 shows the structure of the major pigments in lac colorant. Laccic acids A, B, and C are known as the major pigments in lac colorant. These compounds have the same basic anthraquinone structure but with different R groups. Three major peaks were detected in the TIC. See Figure 11. Although laccic acids A, B, and C were identified, A and B could not be separated.

Figure 12 shows the mass spectra of two peaks, laccic acid C and a combination of laccic acids A and B. The deprotonated molecular ions were observed at m/z 495, 536, and 538. Fragment ions resulting from the loss of carbon dioxide were observed at m/z 451, 492, and 494.

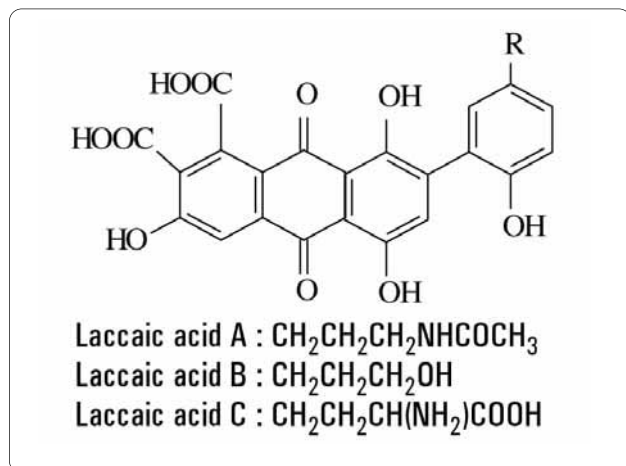


Figure 10. The structure of major pigments of lac colorant.

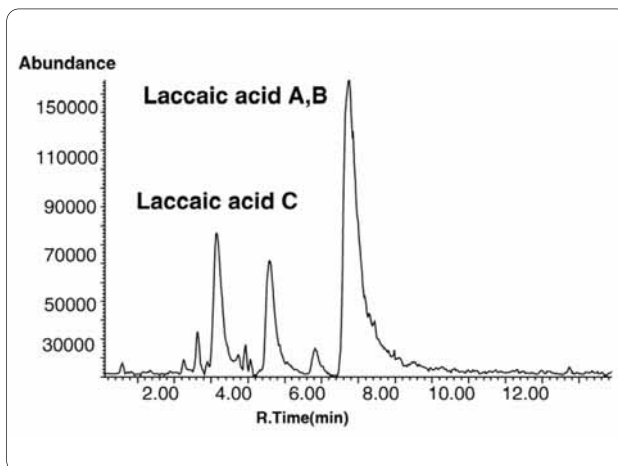


Figure 11. The total ion chromatogram of lac colorant.

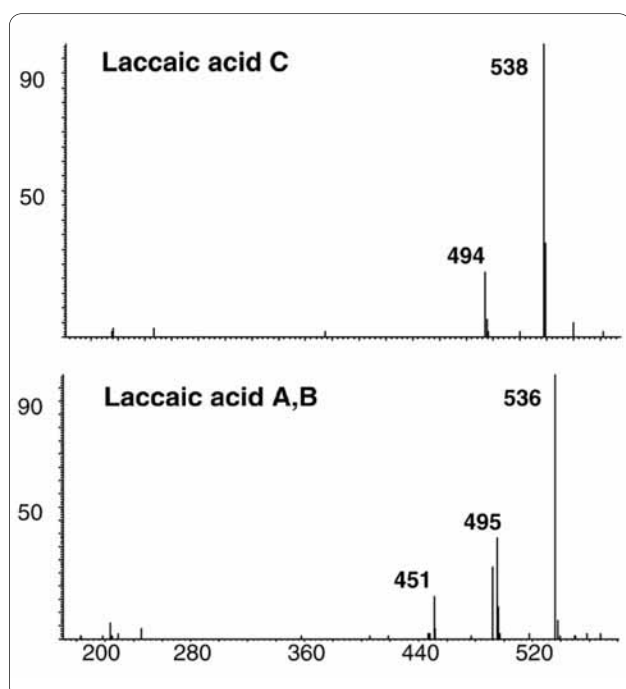


Figure 12. Mass spectra of major pigments in lac colorant.

LC Conditions

Column: Inertsil ODS3, 250 mm × 2.1 mm, 5 μm
 Mobile phase: 30% acetonitrile in 5 mM dibutylamine, isocratic
 Flow rate: 0.2 mL/min
 Column temperature: 40 °C
 Injection volume: 10 μL

MS Conditions

Source: ESI
 Ion mode: Negative
 V_{cap} voltage: 4000 V
 Nebulizer: 50 psig
 Drying gas flow: 10 L/min
 Drying gas temp: 350 °C
 Scan range: 100–1200 amu
 Step size: 0.1
 Peak width: 0.15 min
 Time filter: On
 Fragmentor: 100 V

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Conclusions

Four commercial natural colorants were analyzed using ESI and APCI-LC/MS. The MS data provided molecular weight information and some structural information for the major pigments

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Printed in the USA
 May 20, 2008
 5989-8442EN



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